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J Anim Sci 1997. 75:3038-3045.

Nitrogen Metabolism and Hormonal Responses of Steers Fed Wheat Silage and Infused with Amino Acids or Casein

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ABSTRACT: Four Holstein steers (159 kg) surgically fitted with abomasal-infusion cannulas were used in a 4 × 4 Latin square study to test amino acid (AA) and casein (CAS) infusions on nitrogen balance and hormonal status of steers consuming vegetative wheat (*Triticum aestivum* L.) silage (12.3% CP). Treatments were 5-d infusions of 1) water (CONT), 2) arginine (ARG; 13.69 g/d), 3) limiting amino acids (LAA, 13.69 g/d arginine + 10.92 g/d histidine + 28.97 g/d lysine + 10.88 g/d methionine + 16.96 g/d threonine, and 4) Na-CAS (300 g/d). Whole blood was collected for plasma AA, growth hormone (GH), insulin, and IGF-I concentrations. Data were analyzed by ANOVA, and the following orthogonal contrasts were used to separate treatment means: CONT vs ARG; ARG vs LAA; and LAA vs CAS. Urinary N increased ($P < .02$) for CAS vs LAA. Arginine increased N retention, as did CAS, compared to LAA. Total plasma essential AA were decreased by arginine. Mean plasma insulin concentrations were increased by CAS ($P < .034$). Arginine increased mean plasma GH levels, but not IGF-I. The CAS treatment increased ($P < .015$) IGF-I levels, but not GH. These data suggest that performance of steers fed wheat silage was limited by duodenal AA flow and that arginine was the first-limiting AA. Casein infusion increased plasma insulin and IGF-I, which would explain the improved growth noted in calves and lambs fed forages supplemented with ruminally undegraded protein.

Key Words: Calves, Wheat Silage, Protein Supplements, Amino Acids, Somatotropin

J. Anim. Sci. 1997. 75:3038–3045

Introduction

Winter annual grass forages may not provide adequate amino acids (**AA**) for growth of young ruminants (Barry et al., 1982; Gill and Beever, 1982). Reports of improved performance in ruminants consuming such forages and supplemented with protein as ruminally undegraded protein (**RUP**) can be found (Barry et al., 1982; Gill and Beever, 1982).

Barry et al. (1982) and Gill and Beever (1982) proposed that increased AA flow to and absorption in the small intestine could explain the RUP responses, and MacRae and Ulyatt (1974) showed increased AA absorption was positively correlated with weight gain. Changing the profile of AA absorbed from one of primarily microbial origin (winter annual forage diets; MacRae and Ulyatt, 1974) to one of dietary and microbial AA has improved N balance (Gill and Beever, 1982). Limiting AA in microbial protein include methionine (Met), lysine (Lys), and threonine (Thr) (Richardson and Hatfield, 1978); arginine (Arg), histidine (His), Lys, leucine (Leu), isoleucine (Ile), valine (Val), and the sulfur AA (Merchen and Titgemeyer, 1992); and Arg, His, Lys, and Met (Storm and Ørskov, 1984). Postruminal provision of these AA has increased N retention and thus could support increased rates of gain (Richardson and Hatfield, 1978).

Barry et al. (1982) suggested that changes in hormonal status from increased or altered balance of AA absorbed could increase BW and protein gain. Abomasally infused Arg (.5 g of Arg-HCl/kg BW) was a growth hormone (**GH**) secretagogue in heifers and wethers (Davenport et al., 1990a,b). If greater AA flow or a specific increase in Arg of dietary origin were provided postruminally, circulating GH concentrations could be elevated, perhaps along with IGF-I, which mediates the effects of GH (Gluckman et al., 1987). Greater AA absorption from the small intestine could

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Received October 2, 1996.

Accepted June 17, 1997.

stimulate anabolism by increasing insulin secretion (Davis, 1972).

This experiment was conducted to determine the effects of increased AA flow to the small intestine or the provision of possible limiting AA on N balance, plasma AA, GH, IGF-I, and insulin concentrations in growing steers consuming wheat silage.

Materials and Methods

Four Holstein steer calves (159 kg) were surgically fitted with abomasal infusion cannulas (Driedger et al., 1970) and allowed to recover before initiation of 4 × 4 Latin square infusion experiment. Surgery and animal care were performed under the guidelines of the University of Georgia, Athens Laboratory Animal Care and Use Committee. Each period consisted of 14 d: a 10-d rest period and a 4-d total fecal and urine collection period. Calves were confined to conventional metabolism crates during collection and kept in comfort stalls (.9 × 1.8 m) when not crated. Steers consumed direct-cut wheat silage ad libitum (22.7% DM, 12.3% CP, 65.8% NDF, 40.4% ADF, 25.4% hemicellulose, 35.7% cellulose, 4.6% lignin, and 7.3% ash) twice daily (0800 and 2000) during each 10-d rest period and 4-d collection period. Water and trace mineralized salt were freely available.

The following abomasally infused treatments were assigned to steers at random and were scheduled through subsequent periods of a 4 × 4 Latin square designed experiment: 1) water (**CONT**); 2) 13.69 g/d Arg (**ARG**); 3) Arg (13.69 g/d) + His (10.92 g/d) + Lys (28.97 g/d) + Met (10.88 g/d) + Thr (16.96 g/d) (limiting AA; **LAA**); and 4) 300 g/d Na-caseinate (**CAS**) (amino acids and CAS from U.S. Biochemical Corp., Cleveland, OH). Individual AA were infused at a rate equal to the amount supplied by CAS infusion.

Infusates (4 L/d) were mixed daily, refrigerated overnight, and infused at ambient temperature during each collection period. The casein solution was prepared by dissolving casein in deionized water with the pH adjusted to 10.0 with NaOH. Infusion, using a constant flow pump (Technicon Instruments, Tarrytown, NY), began at 1200 on d 1 of each collection period and was maintained continuously for 5 d.

On d 1 to 4, total fecal output was collected, weighed, and a 2% subsample was collected. Subsamples were dried (55°C) to a constant weight, composited by steer and period, and ground in a Wiley mill (1-mm screen, Arthur A. Thomas, Philadelphia, PA). Dry matter (DM) and ash were determined using AOAC (1990) procedures. Nitrogen was measured on an Autoanalyzer (Model II Technicon Instruments) after digestion on a block digester (AOAC, 1990). Urine was preserved in 6 *N* HCl (100 mL) during daily collections. Volume was measured and a 1% subsample collected. Composites were made by steer and period and frozen until analyzed for N as described above.

Feed refusals were collected and weighed daily. Orts and daily grab samples of forage were dried (55°C) to a constant weight, ground through a Wiley mill, and analyzed for DM, ash, and N as described previously. Amino acid profiles of silage and casein were determined as given by Amos et al. (1976) using a Beckman 6300 Amino Acid Analyzer (Beckman Instruments, Palo Alto, CA).

Jugular catheters were fitted in each steer at 1900 on d 2 of each collection period. On d 3, 10-mL blood samples were drawn from each steer at 0800, 1200, 1600, and 2000 into tubes containing 75 μL of a 15% EDTA solution for AA analysis (Borum, 1988). An equal amount of sterile physiological saline was infused via the catheter after each blood sample to maintain blood volume. Blood samples were placed in ice immediately after collection, refrigerated at 4°C for 24 h, and plasma was collected after centrifugation (3,000 × *g*) for 20 min. Plasma was deproteinized with sulfosalicylic acid (5%) and the deproteinized plasma was separated by centrifugation (5,000 × *g*). Equal aliquots of deproteinized plasma were composited and free AA were determined using a lithium citrate buffer system (Beckman 6300 AA Analyzer, Beckman Instruments).

On d 4, 3-mL blood samples were collected into tubes containing 25 μL of a 15% EDTA solution every 10 min from 0800 to 1600. At 1600, Arg (.5 g/kg BW; Elsasser et al., 1986) was infused into the jugular catheter to induce GH release and blood samples were collected every 10 min for an additional hour. Blood samples were chilled and centrifuged (3,000 × *g* for 20 min). The supernatant was frozen (−20°C) until it was analyzed for GH and insulin as described by Sartin et al. (1985). Insulin analyses were conducted on samples collected at 0800 and 1600 (only treatment means are reported). Intra- and interassay CV for GH were 6.8% and 8.0%, and for insulin, 10.2% and 13.3%, respectively. The minimum detectable concentrations were 2 ng/mL for GH and 4 μU/mL for insulin.

On d 4, additional blood samples were collected from each steer at 30-min intervals from 0800 to 1600 and allowed to clot. After centrifuging (3,000 × *g* for 20 min), the serum was removed and frozen (−20°C) until it was analyzed for IGF-I as described by Houseknecht et al. (1988). The intraassay CV was 3.5% and the interassay CV was 4.8%. The minimum detectable concentration of IGF-I was 14 ng/mL.

Nitrogen balance, insulin, basal and mean GH concentrations, GH peak amplitude, peak interval and number of GH pulses, the insulin:GH ratio, and AA plasma concentrations were analyzed using the GLM procedure of SAS (1985). For each steer, mean GH concentrations, basal GH concentrations, number of GH pulses, and GH pulse amplitude were determined by PULSAR analysis using a 1% criterion of variation

Table 1. Nitrogen balance of steers fed wheat silage and abomasally infused with water (CONT), arginine (ARG), five potentially limiting amino acids (LAA), or casein (CAS)

	Infusate					Contrast[a]		
Item	CONT	ARG	LAA[b]	CAS	SE	1	2	3
N intake, g/d	61.1	67.3	70.6	102.8	2.1	.080	.301	.001
Fecal N, g/d	29.5	29.4	27.6	29.0	1.0	.968	.261	.390
Urinary N, g/d	38.0	35.4	37.4	49.5	2.6	.508	.617	.018
N digestibility, %	51.6	55.5	60.7	71.8	1.5	.113	.044	.002
N retained, g/d	–6.4	2.5	5.7	24.4	2.8	.066	.451	.003
N retained, % intake	–11.3	2.1	6.3	23.0	3.8	.046	.463	.021
N retained, % digested	–23.6	2.8	9.4	32.1	7.2	.042	.537	.068

[a]Probabilities of contrasts: 1 = CONT vs ARG; 2 = ARG vs LAA; 3 = LAA vs CAS.
[b]LAA = Arg, His, Lys, Met, Thr.

(Merriam and Wachter, 1982). Mean IGF-I and insulin concentrations were subjected to a split plot in time ANOVA using the GLM procedure of SAS (1985). The main effects of steer, period, and treatment, as well as the contrasts listed below, were tested using the treatment × steer (period) interaction as the error term (Steele and Torrie, 1980). The following preplanned contrasts were used to separate differences in treatment means: CONT vs ARG; ARG vs LAA; and LAA vs CAS.

Results and Discussion

Nitrogen Balance. Forage DM intake (CONT, 3,099; ARG, 3,178; LAA, 2,886; and CAS, 3,071 g/d; SE, 97.4) differed only between ARG and LAA ($P < .08$). According to the NRC (1984), this DM intake would provide 77.7, 80.0, 73.1, and 77.7% of the daily DM recommendation for a medium-frame steer gaining .23 kg for CONT, ARG, LAA, and CAS, respectively. This level of silage DM intake also provided approximately 97.8, 100.7, 92.0, and 97.8% of the daily TDN recommendations, where TDN = 105.2 – .68 (% NDF) (D. R. Mertens, personal communication) and 103.8, 106, 96.5, and 103% of the daily CP recommendations (NRC, 1984) for CONT, ARG, LAA, and CAS, respectively. The reason for lowered forage DM intake in calves receiving the LAA treatment is unclear, but the lowered intake explains why ARG-infused and LAA-infused steers were similar in total N intake.

Steers were supplied with an additional equivalent of 22.8, 94.6, and 270.0 g of CP/d by ARG, LAA, and CAS infusates, respectively. Thus, total CP intake was 103.6, 113.7, 123.7, and 174.7% of NRC (1984) dietary recommendations for a steer gaining .23 kg/d. Infusing 13.69 g/d of Arg increased ($P < .08$) N intake compared with CONT (Table 1). The ARG- and LAA-treated steers did not differ, but steers infused with casein had greater N intakes than steers infused with LAA ($P < .001$). These differences are primarily due to the added N in the respective infusates; forage DM intake differed only between ARG and LAA ($P < .08$).

Fecal N did not vary among treatment groups, which is consistent with other studies (Gow et al., 1979; Koenig et al., 1982; Davenport et al., 1990b) in which Arg infusion, even at higher levels of administration (e.g., .5 g Arg-HCl/kg BW), also did not affect fecal N. Fecal N also remained unchanged with infusions of other AA, such as Met (Titgemeyer and Merchen, 1990) and Lys (Burris et al., 1976) and casein (Gow et al., 1979). Apparent digestibility of N was increased in steers receiving LAA compared to Arg alone and by casein infusion compared with LAA. Because fecal N did not increase with greater N input for the LAA and CAS treatments, the treatments improved apparent total tract N digestibility by being essentially completely absorbed.

Urinary N was similar among steers receiving the CONT and ARG infusions and between those infused with ARG and LAA. Previous Arg infusion studies with heifers (Koenig et al., 1982; Davenport et al., 1990a) and wethers (Davenport et al., 1990b) resulted in increased urinary N excretion over controls. However, higher levels of Arg (.5 g Arg-HCl/kg BW; 6.4 g of N/d, respectively) were used in the earlier studies. It should also be noted that the 1-d adjustment period to infusates was minimal and that some of the increased N balance may represent increases in the N content of intracellular and extracellular fluid. Biddle et al. (1975) showed that the plasma urea concentration increased almost immediately as nitrogen intake increased. Casein infusion increased urinary N ($P < .02$) in the current study. This increased urinary N from steers infused with CAS probably reflects the high total N input (approximately 1.75 × recommendations; NRC, 1984) and marginal TDN intake (approximately 98% of NRC [1984] recommendations) noted earlier. Overall, these data suggest that suboptimal TDN intake (energy) limited N utilization in steers infused with CAS. The actual CP intake in CAS treatment provided CP for almost 1.1 kg of daily gain; whereas TDN was adequate for less than .23 kg of daily gain. The increased urinary N excretion by steers infused with CAS suggests that amino acids in excess of requirements may have been used for gluconeogenesis or in energy metabolism.

Table 2. Amino acid inputs of steers fed wheat silage and abomasally infused with water (CONT), arginine (ARG), five potentially limiting amino acids (LAA), or casein (CAS)

	Infusate					Contrast[a]		
Amino acid	CONT	ARG	LAA[b]	CAS	SE	1	2	3
	g/d							
Arg	4.8	16.3	15.8	16.3	.18	.001	.060	.087
His	2.8	3.0	10.8	11.9	.11	.421	.001	.001
Ile	14.2	14.7	13.3	30.9	.52	.466	.094	.001
Leu	24.8	25.7	23.2	54.5	.87	.490	.001	.001
Lys	6.7	7.0	29.5	30.9	.25	.428	.001	.010
Met	6.4	6.7	16.9	15.5	.23	.490	.079	.005
Phe	13.0	13.4	12.1	29.4	.44	.521	.001	.001
Thr	8.2	8.7	24.7	22.3	.36	.379	.092	.003
Val	18.2	18.9	17.1	39.1	.66	.471	.001	.001
EAA[c]	99.2	114.4	163.4	250.7	3.59	.024	.001	.001
Ala	33.8	35.5	31.7	43.5	1.37	.421	.099	.001
Asp	13.3	13.9	12.5	36.9	.50	.444	.099	.001
Cys	0	0	0	1.5	0	0	0	0
Glu	24.9	25.9	23.4	100.6	.91	.460	.098	.001
Gly	14.6	15.3	13.7	20.7	.58	.425	.095	.001
Pro	9.7	10.3	9.2	47.5	.42	.379	.119	.001
Ser	7.1	7.4	6.7	25.9	.28	.408	.130	.001
Tyr	4.2	4.3	3.9	22.7	.15	.496	.074	.001
NEAA[d]	107.5	112.5	101.0	299.2	4.18	.427	.099	.001

[a]Probabilities of contrasts: 1 = CONT vs ARG; 2 = ARG vs LAA; 3 = LAA vs CAS.
[b]LAA = Arg, His, Lys, Met, and Thr.
[c]EAA = total essential amino acids.
[d]NEAA = total nonessential amino acids.

Steers receiving the CONT treatment were in negative N balance (Table 1). Although fecal and urinary N were not different between CONT and ARG-infused steers, their sum resulted in more ($P < .066$) N retained by the steers infused with ARG. Steers receiving the ARG and LAA treatments did not differ even though N balance increased from 2.5 to 5.7 g/d. The CAS infusion compared to LAA resulted in an 18.7 g/d increase in N retention and suggests an improved balance of metabolizable AA. Steers infused with CAS received 41.7 g daily more N and retained 30.8 g more N than those receiving the CONT infusion. The increases in daily N retained as a percentage of the increase in N intake for ARG compared with CONT indicates 144% of the increased N intake was retained, compared with 127% for LAA vs CONT and 74% for CAS vs CONT. These latter data were calculated as the absolute difference in N retained for ARG, LAA, and CAS compared to CONT divided by the increase in N intake of each respective treatment. Fecal N was 29.0 compared to 29.5 for steers receiving CAS and CONT treatments, respectively, and urinary N increased by 11.5 g in steers receiving CAS vs CONT. These results suggest that the N requirement was met or exceeded by CAS and that energy or some other nutrient became limiting.

Overall, nitrogen metabolism results indicate that the forage diet alone was unable to provide adequate AA for growth. The positive N balance response to ARG indicates that Arg was the first-limiting AA, although its influence on hormonal status cannot be overlooked. Because Arg seems to be the first-limiting AA, this response may be related to a study by Chalupa (1975), who reported that Arg was the most extensively degraded AA either as a free AA or in alfalfa by ruminal microorganisms during in vitro fermentation. Williams et al. (1995) also reported that Arg was the most extensively degraded AA (approximately 60%) during ensiling of wheat forage. Postruminal provision of a complete protein (CAS) clearly produced the greatest N retention response, as previously noted when one or a few AA infused into growing steers were compared with infusion of intact protein (Merchen and Titgemeyer, 1992).

Amino Acids. As shown in Table 2, the ARG treatment provided greater Arg ($P < .001$) and total essential AA (**EAA**; $P < .03$) inputs compared with CONT. More His, Lys, and Thr were provided by LAA infusate than ARG infusate. However, due to greater forage DM intake by steers infused with ARG, the ARG-infused steers consumed more Arg, Ile, Leu, Phe, Val, total EAA, and total nonessential AA (**NEAA**). Casein uniformly increased input of all AA, except Cys, compared with LAA. Infusates increased total EAA intakes in all cases.

Plasma Arg concentrations (Table 3) were similar for steers infused with ARG and those infused with CONT, even though Arg intake increased by 11.5 g/d for steers receiving the ARG treatment. Contrary to other results from growing steers fed a purified diet (Richardson and Hatfield, 1978), these data indicate that Arg was first-limiting, although use of plasma AA

Table 3. Venous plasma amino acid concentrations of steers fed wheat silage and abomasally infused with water (CONT), arginine (ARG), five potentially limiting amino acids (LAA), or casein (CAS)

	Infusate					Contrast[a]		
Amino acid	CONT	ARG	LAA[b]	CAS	SE	1	2	3
	μM/dL							
Arg	18.1	17.1	21.7	19.1	.18	.509	.019	.125
His	9.0	8.3	11.1	9.8	.11	.290	.003	.052
Ile	8.4	5.9	4.9	12.0	.52	.095	.472	.001
Leu	9.9	7.3	4.2	16.0	.87	.115	.066	.001
Lys	9.8	7.2	18.4	12.3	.25	.119	.001	.005
Met	2.1	1.4	4.2	3.0	.23	.143	.001	.028
Phe	4.3	3.3	3.1	5.3	.44	.022	.618	.001
Thr	5.5	4.1	19.2	6.2	.36	.436	.001	.001
Val	17.8	13.8	9.4	28.1	.66	.160	.115	.001
EAA[c]	84.8	68.5	96.1	111.6	3.59	.104	.018	.119
Ala	14.5	12.9	16.4	14.9	1.37	.252	.130	.318
Asp	0	0	0	0	.50	0	0	0
Cys	.6	.7	.8	.7	0	.411	.044	.115
Glu	11.9	11.0	10.2	11.4	.91	.298	.338	.169
Gly	38.4	32.6	35.4	30.6	.58	.024	.194	.048
Pro	0	0	0	.4	.42	1.000	1.000	.055
Ser	13.6	12.4	11.5	12.7	.28	.172	.310	.163
Tyr	3.0	1.9	1.7	5.2	.15	.138	.830	.002
Orn	6.7	9.1	6.7	7.2	.53	.020	.021	.589
NEAA[d]	82.1	71.4	76.1	76.0	4.18	.020	.216	.988

[a]Probabilities of contrasts: 1 = CONT vs ARG; 2 = ARG vs LAA; 3 = LAA vs CAS.
[b]LAA = Arg, His, Lys, Met, and Thr.
[c]EAA = total essential amino acids.
[d]NEAA = total nonessential amino acids.

to determine limiting AA is problematic due to the effects of AA turnover and flux on the free AA pool (Bergen, 1979). Further evidence may be provided by the drop ($P < .104$) in total plasma EAA for ARG-infused steers (68.5 μmol/dL) compared with CONT-infused steers (84.8 μmol/dL). Also, postruminal provision of the first-limiting AA decreases urinary N and improves N retention (Nimrick et al., 1970), as occurred in this experiment (Table 1).

Although lower energy intakes by the steers infused with LAA may also be involved in this response, the increase ($P < .02$) in plasma Arg concentration noted for steers infused with LAA compared with ARG-infused steers and a trend toward decreased ($P < .125$) plasma Arg when CAS and LAA were compared indicate that an AA other than His, Lys, Met, or Thr was second-limiting. This is supported by uniform increases in plasma concentrations of these AA when LAA and ARG are compared and indicates that the amounts of these AA infused were greater than the requirements for tissue protein synthesis (Richardson and Hatfield, 1978). Decreases in plasma His, Lys, Met, and Thr and increased percentage of intake and percentage of digested N retained ($P < .02$ and $P < .07$, respectively) when CAS was infused compared with LAA indicate increased tissue AA uptake when CAS was infused. The N balance data also support Arg as the first-limiting AA, followed by a second-limiting AA not provided in the LAA treatment, because improvements in N retention only occurred with ARG compared with CONT and with CAS compared with LAA. However, calculating an index of plasma AA (Potter et al., 1966) by expressing each essential AA in the plasma of steers infused with Arg as a percentage of CONT shows that plasma Met concentration decreased by 33.4% compared to decreases of 5.5, 7.8, 29.8, 26.3, 26.6, 23.3, 25.5, and 22.5 for Arg, His, Ile, Leu, Lys, Phe, Thr, and Val, respectively. Conversely, plasma AA concentration of the specific AA in LAA increased significantly but plasma concentrations of Ile, Leu, and Val decreased by 41.7, 57.6, and 47.2% compared to ARG. These data suggest that Met and Leu may have been second- and third-limiting AA in steers fed wheat silage in the current study.

Hormonal Status. Mean plasma concentrations of insulin (Table 4) were not affected by treatment, except for CAS vs LAA. The increased ($P < .03$) insulin concentration induced by casein infusion has been reported previously when 300 g/d casein was abomasally infused into beef steers consuming a high-concentrate diet (Guerino et al., 1991) and when 44 g/d Na-caseinate + .5 g/d Met were abomasally infused into growing lambs consuming ryegrass pasture (Barry et al., 1982). Steers infused with CAS received 41.7 g/d more N than the CONT steers, retained 30.8 g/d more N, and excreted 11.5 g/d more urinary N, indicating that some of the infused AA may have been used for gluconeogenesis. However, it is doubtful that this source of glucose from AA would have influenced

Table 4. Analysis of insulin (INS), growth hormone (GH), and insulin-like growth factor-I secretion in steers fed wheat silage and abomasally infused with water (CONT), arginine (ARG), five potentially limiting amino acids (LAA), or casein (CAS)

	Infusate					Contrast[a]		
Item	CONT	ARG	LAA[b]	CAS	SE	1	2	3
Mean INS, μU/mL	5.6	5.5	5.9	8.8	.74	.947	.677	.034
Mean GH, ng/mL	12.4	19.6	7.5	7.8	1.26	.007	.001	.889
Basal GH, ng/mL	9.2	12.8	4.8	5.3	1.34	.104	.005	.805
GH peak amplitude, ng/mL	7.7	19.7	5.8	7.4	2.37	.012	.006	.652
GH peak length, min	60.4	97.0	63.8	59.1	12.71	.088	.114	.806
GH peaks, no./8 h	3.8	3.5	5.5	4.8	.41	.680	.013	.242
Mean IGF-I, ng/mL	46.3	46.4	53.6	74.7	2.38	.988	.292	.015

[a]Probabilities of contrasts: 1 = CONT vs ARG; 2 = ARG vs LAA; 3 = LAA vs CAS.
[b]LAA = Arg, His, Lys, Met, and Thr.
[c]EAA = total essential amino acids.
[d]NEAA = total nonessential amino acids.

insulin secretion markedly. Also, casomorphins, or peptides from casein that are resistant to pepsin hydrolysis, have been shown to stimulate insulin secretion (Paroli, 1988).

Casein infusion had no effect on mean or basal plasma GH levels compared with LAA (Table 4), but ARG infusion increased plasma GH concentrations compared with CONT ($P < .007$) and LAA ($P < .001$). These values seem to have resulted from increased peak amplitude and length when ARG was infused. Reports of the effects of abomasally infused Arg on GH levels suggest that GH release is dependent on amount and rate of L-Arg infused (threshold response) under certain experimental conditions. Infusions of .5 g/d Arg-Hcl/kg BW to heifers (Davenport et al., 1990a) and wethers (Davenport et al., 1990b) resulted in increased mean and basal GH levels, whereas .25 g/d L-arg-HCl/kg BW did not (Davenport et al., 1990a). In addition, 25 g/d (approximately .625 $g \cdot kg\ BW^{-1} \cdot d^{-1}$ (Gow et al., 1979) abomasally infused into lactating goats and 178 g/d (approximately .32 g/kg BW) abomasally infused into lactating dairy cows (Vicini et al., 1988) did not increase GH, but infusion of .1 g/kg BW of Arg-HCl twice daily (approximately 55 g/time infused over 5 min) increased GH and insulin (Vicini et al., 1988). Differences in productive status of the experimental animals (lactation vs growth), the need for Arg for milk protein synthesis, and L-Arg administration rate probably explain variability of results in different studies.

Steers receiving the LAA (7.21 ng/mL, SD = 1.16) and CAS (12.33 ng/mL, SD = 1.80) infusions had decreased capacity to release GH in response to an intravenous Arg challenge (.5 g/kg BW) compared with CONT steers (21.3 ng/mL, SD = 2.21) and steers treated with ARG (21.67 ng/mL, SD = 2.36). Responses between ARG- and LAA-treated steers differed ($P < .005$); however, responses between CONT and ARG ($P > .93$) and between LAA and CAS ($P > .17$) did not differ. Houseknecht et al. (1988) also reported a greater ability to release GH in response to intravenous Arg infusion by heifers (212 kg) consuming low-energy (soyhull and soybean meal) wheat silage-based diets compared to heifers receiving higher-energy (corn, soyhulls, and soybean meal) wheat silage-based diets.

Because GH responses to intravenous Arg infusions are delayed, Arg was proposed to act as a GH secretagogue through a metabolite (Vicini et al., 1988). Ornithine or one of its metabolites may be the secretagogue in question; Davenport et al. (1990b) demonstrated ornithine and Arg were equally capable of increasing serum GH concentrations. Plasma ornithine (Table 3) concentrations were higher ($P < .02$) in calves infused with ARG (9.1 mg/dL) than in those receiving the CONT infusion (6.7 mg/dL) and when ARG was compared with LAA ($P < .02$; 6.8 mg/dL) in this study. This may indicate that the 13.69 g/d of Arg supplied was used for protein synthesis, as evidenced by the N retention data, and to generate ornithine for the urea cycle. This dual role for Arg may have been accentuated due to the limitations placed on protein synthesis by the absence of the next limiting AA and subsequent excess of available Arg.

The drop in serum ornithine and GH levels that occurred when steers were given Arg in combination with His, Lys, Met, and Thr or as part of the casein infusion indicates that Arg was not used as a GH secretagogue in these treatments. The increased ($P < .003$) daily N retained of steers receiving the CAS treatment indicates that Arg was preferentially used for protein synthesis and as a result increased N retention without increasing plasma GH, as noted with ARG.

It is interesting to note that the high level of circulating GH on the ARG treatment was not associated with the largest increase in N retention. However, mean serum IGF-I values (Table 4) are more reflective of the N balance data; CAS-infused steers expressed the greatest increase in serum IGF-I concentrations and N retention. These data are contrary to a previous report of no effect of a

3-d, 300 g/d casein infusion on arterial IGF-I concentrations (Guerino et al., 1991). Insulin-like growth factor I is proposed to mediate the actions of GH on tissues (Gluckman et al., 1987), and measures of circulating concentrations of IGF-I have been positively correlated with growth in young ruminants (Olsen et al., 1981). Thus, the increase in N retention observed in steers receiving the CAS treatment is reflective of serum increased IGF-I levels, even without a concomitant increase in plasma GH. This lack of GH increase and increase IGF-I in steers receiving CAS may be due to a negative feedback of IGF-I or GH release, as was shown for rat pituitary slices incubated in vitro with IGF-I or GH release, as was shown by Yamashita and Melmed (1986). Additionally, small peptides in β-casein that exert opioid activity known as casomorphins have been shown to stimulate secretion of somatostatin, a negative regulator of GH secretion (Paroli, 1988), and would keep circulating GH levels low in steers receiving CAS compared with those receiving the non-casein treatments.

The increase in circulating GH concentrations in ARG-infused steers, without an accompanying increase in plasma IGF-I or a large increase in N retention, may be explained by GH resistance, as proposed by Gluckman et al. (1987) for ruminants on pasture. Those authors suggested that such animals, consuming less than optimal levels of DM, release IGF-I in response to GH less efficiently than animals with greater DM intakes. Underfed ruminants have exhibited increased GH half-life and lower turnover and clearance rates compared with adequately fed counterparts (Trenkle, 1976). Apparently, under a physiological state of negative energy balance, tissues become refractory to GH, resulting in GH resistance. Results of the current study are similar; animals on a lower plane of nutrition had higher concentrations of plasma GH without accompanying increases in plasma IGF-I. Provision of LAA or CAS apparently reduced the tissue refractoriness, increasing plasma GH efficiency at stimulating IGF-I release and, for CAS, increasing N retention.

The data collected indicate that performance of growing steers consuming wheat silage alone is limited and Arg seems to be the first AA limiting performance. Probable candidates as second-limiting AA include either Met or Leu. Improved N retention on the CAS treatment indicates that steers benefited most from an intact protein that provides a greater supply or broader spectrum of AA to the small intestine. Clearly, increasing the postruminal supply of AA and protein affected the hormonal status of growing steers. Although Arg alone increased plasma GH levels, a similarly large increase in protein anabolism did not occur, possibly due to GH resistance or to the absence of lesser order limiting AA needed to support protein synthesis. The increase in plasma insulin and IGF-I, which accompanied CAS infusion, may be critical in generating the increased N retention noted in steers on this treatment.

Implications

Growing ruminants consuming high-protein grass forages seem to benefit from supplemental protein through provision of greater duodenal amino acid flow and a subsequent increase in anabolic hormone levels. Further research is needed to determine whether proteins other than casein are capable of stimulating the insulin and(or) insulin-like growth factor I responses noted in this study. The order of limiting AA for growth of ruminants fed wheat silage should include arginine, methionine, and leucine. Additionally, the benefit of arginine-induced increases in plasma growth hormone for forage-fed animals should be investigated further.

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